

Short Communication

ISOLATION OF *Moraxella bovis* FROM INFECTIOUS KERATOCONJUNCTIVITIS IN A FLOCK OF GOATS

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INTRODUCTION

Infectious keratoconjunctivitis (IKC) is a highly contagious ocular disease of ruminants which may lead to temporary or permanent blindness in affected animals (Brian *et al.*, 2006). The condition is worldwide in distribution and has been reported in different parts of the world including North America, Europe, Asia, Africa and Australia (Degiorgis *et al.*, 2000; Giacometti *et al.*, 2002). Generally at onset, IKC is characterized by unilateral or bilateral inflammation of the conjunctiva and cornea with moderate to severe hyperaemia, epiphora, blepharospasm and photophobia (Bankemper *et al.*, 1990). If untreated, these progress to mucopurulent ocular discharge with matting of hair around the eyes, the cornea becomes opaque and may be perforated in advanced stages (Bankemper *et al.*, 1990). The following pathogens have been implicated in the aetiology of IKC in goats: *Mycoplasma spp.*, *Branhamella spp.*, *Chlamydia spp.*, *Rickettsia spp.*, *Corynebacterium pyogenes*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Streptococcus spp.*, *Pseudomonas spp.* (Åkerstedt and Hofshagen 2004). The involvement of each of these organisms in the pathogenesis of IKC varies considerably. Some are major and common cause of IKC while others are mostly present as opportunistic invaders which may complicate the condition (Åkerstedt and Hofshagen, 2004). This study investigates an outbreak of IKC in a flock of intensively managed goats with a view to identifying the bacterial pathogens associated with the disease.

KEY WORDS: Infectious keratoconjunctivitis, goat, *Moraxella bovis*, *Mycoplasma spp.* and *Staphylococcus aureus*

FLOCK HISTORY

An outbreak of eye disease manifesting as keratoconjunctivitis occurred in a flock of goat belonging to the University of Agriculture, Abeokuta. The goats were kept primarily for experimental purposes and partly for income generation. During this study, there was no experiment being carried out on the flock. About 56 goats comprising of 40 does and 16 bucks were present at the time of this study. The flock comprised of two major breeds of goats which were the West African Dwarf (WAD) and

Red Sokoto (RS) as well as cross between the two. The age of the animals ranged from 2 weeks to 7 years.

MICROBIOLOGICAL INVESTIGATION

Sample collection

Ocular discharges were collected directly from both eyes of clinically affected animals and also from eyes of apparently healthy animals in the flock using sterile swabs. Eight samples were collected from four clinically affected animals and another eight from four apparently healthy

animals making a total of sixteen samples. Each sample swab was inserted into sterile distilled water in universal bottle and preserved with ice-pack in a cooler. The samples were labeled appropriately and promptly transported to Microbiology laboratory of the Faculty of Veterinary Medicine, University of Ibadan where they were bacteriologically examined.

Laboratory procedures

Giemsa-stained direct smear were first made from all samples and studied microscopically for the presence of chlamydiae. The samples were then each inoculated onto blood agar (Oxoid[®] blood agar base enriched with 5% sheep blood), MacConkey agar (Oxoid[®]) as well as onto Mycoplasma agar 'P' (Oxoid[®]) to which Mycoplasma supplement (Oxoid[®]) was added. Inoculated blood agar and MacConkey agar plates were incubated aerobically at 37°C for 24 to 48 hours while the inoculated Mycoplasma agar plates were incubated at 37°C in CO₂ incubator at an atmosphere of 10% CO₂ for 10 to 14 days.

Bacterial identification and characterization

Following incubation, agar plates were observed for bacterial growth. The characteristics of observed colonies were studied. Other identification tests carried out included: morphological studies by microscopy; motility, catalase, oxidase, sugar fermentation, haemolysis on blood agar, gelatin liquefaction and coagulase as described by Barrow and Feltham (1993).

Pathogenicity testing

Pathogenicity of pure isolates of *Mycoplasma spp* recovered from clinical samples was tested in two guinea-pigs by intraocular instillation of broth culture of the organism.

Antimicrobial Sensitivity Testing

The disk diffusion method on Mueller-Hilton agar (Oxoid[®]) was employed to test the antimicrobial susceptibility of the isolates (NCCL, 1997). Susceptibility to the following antimicrobial agents was determined: Gentamycin (10ig), nitrofurantoin (300ig), ofloxacin (30ig), ampicillin (25ig), streptomycin (10ig),

erythromycin (5ig), cloxacillin (5ig), cotrimoxazole (25ig), penicillin G (10 units) and chloramphenicol (20ig) (Oxoid[®]).

RESULTS AND DISCUSSION

Case presentation

In this outbreak, the initially signs observed were unilateral hyperemia of the conjunctiva and some level of mucopurulent ocular discharges. However, two days later the lesion became bilateral and discharges copious with matting of hair around the eye. There was also cornea opacity resulting in blindness (Fig. 1). Four (7.14%) out of 56 goats in the flock showed clinical signs of IKC. All affected goats were WAD breed and between 2 and 3 years old.



FIGURE 1: Eye of a goat with keratoconjunctivitis showing inflamed conjunctiva, cornea opacity and matting of hair around the eye

Bacterial isolates

Moraxella bovis was isolated from all the eight (100%) clinical samples as well as from one (12.5%) of the eight non-clinical samples. However, coagulase positive *Staphylococcus aureus* was isolated from all the sixteen (100%) clinical and non-clinical samples. *Mycoplasma*

spp was also isolated from one (12.5%) of eight clinical samples but not from any of the eight non-clinical samples.

Bacterial characteristics

Staphylococcus aureus: catalase positive, oxidase negative, coagulase positive, maltose positive, mannitol positive, non-motile, Gram-positive cocci that produced α -haemolytic yellowish colonies on blood agar.

Moraxella bovis

Catalase positive, oxidase positive, non-motile, non-saccharolytic, proteolytic (on nutrient gelatin slope), auto-agglutinating, Gram-negative short, plump rods that produced α -haemolytic, embedding, shiny colonies on blood agar but no growth on MacConkey agar.

Mycoplasma spp

Urease negative, digitonin sensitive bacterium, with producing very small colonies with fried-egg appearance on agar when observed using dissecting microscope.

The isolation of *Moraxella bovis* from all the clinical cases but *Mycoplasma spp* from just one of these cases strongly implicated *Moraxella bovis* as the primary pathogen responsible for the clinical condition in the affected animals. It appears *Mycoplasma spp* is an opportunistic pathogen while the presence of *Staphylococcus aureus* in both diseased and apparently healthy animals might have predispose to infection. *Mycoplasma conjunctivae* is a common cause of IKC in sheep and goat (Giacometti *et al.*, 2002). However, the species of *Mycoplasma* isolate in this investigation was not determined but its pathogenic significance was studied by experimental conjunctival inoculation into guinea pig; a widely used laboratory animal in microbiological investigations. Experimental instillation of the *Mycoplasma spp* isolated in the present study failed to induce IKC in guinea pigs. The present case is attributed to *Moraxella bovis* (an uncommon cause of IKC in goats) because of its presence in all the clinical cases

and not to *Mycoplasma* which was present only in one of the clinical cases. The goat flock investigated in the present study was situated on the farm not far from a cattle herd. The goats might have acquired *Moraxella bovis* from cattle (the major host of *Moraxella bovis*). Concurrent viral infections, malnutrition, parasitic infestations, extreme weather and other stressful conditions leading to immunosuppression could have increase the susceptibility of the goats to *Moraxella bovis* and contributed to the precipitation of IKC in the affected goats.

Antimicrobial sensitivity

Both *Moraxella bovis* and *Staphylococcus aureus* isolated in this study were resistant to ampicillin (25ig), streptomycin (10ig), erythromycin (5ig), cloxacillin (5ig), cotrimoxazole (25ig), penicillin G (10 units) and chloramphenicol (20ig) (Oxoid[®]). They were susceptible only to gentamycin, nitrofurantoin and ofloxacin. Antimicrobial resistance could be due to indiscriminate and inappropriate use of antimicrobial agents in livestock.

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